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Beneficial influence of dietary curcumin, capsaicin and garlic on erythrocyte integrity in high-fat fed rats

Rayavara K. Kempaiah, Krishnapura Srinivasan*

Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore-570 020, India

Abstract

In rats rendered hyperlipidemic by maintaining them on a high-fat diet (30%) for 8 weeks, inclusion of spice principles [curcumin (0.2%) or capsaicin (0.015%)] or garlic (2.0%) in the diet produced significant hypotriglyceridemic effect. Plasma cholesterol remained unaffected in high-fat treatment. Hepatic triglyceride content was significantly higher in high-fat fed rats, and this increase was effectively countered by inclusion of the hypolipidemic spice agents — curcumin, capsaicin or garlic in the diet. The lipid profile of erythrocyte membranes of hyperlipidemic rats was similar to basal controls. An examination of the osmotic fragility of erythrocytes in various groups indicated that the red blood cells of hyperlipidemic rats display a slight resistance to osmotic lysis. Inclusion of spice principles [curcumin (0.2%) or capsaicin (0.015%)] or garlic (2.0%) in the diet, which produced the hypotriglyceridemic effect, appeared to beneficially correct this altered osmotic fragility of erythrocytes. Activities of ouabain-sensitive Na^+,K^+-ATPase as well as acetylcholinesterase of erythrocyte membranes in high-fat fed rats remained unaltered. Activity of Ca^{2+},Mg^{2+}-ATPase in erythrocyte membrane was significantly decreased in high-fat fed animals, whereas dietary spice principles and garlic countered this reduction in enzyme activity. In the absence of any change in the cholesterol/phospholipid molar ratio in the erythrocyte membrane, a decreased activity of membrane-bound Ca^{2+},Mg^{2+}-ATPase could have probably contributed to the accumulation of intracellular calcium leading to the diminished deformability of the erythrocytes in high-fat fed rats.

Keywords: Dietary spices; High-fat diet; Hypertriglyceridemia; Erythrocyte integrity

1. Introduction

Spices that are consumed as food adjuncts to enhance sensory quality of foods impart characteristic flavor, aroma and color to foods. Besides, spices also possess several medicinal properties and hence find application in the indigenous systems of medicine. During the past three decades, several beneficial physiological effects of spices have been experimentally documented, of which the hypolipidemic potential of a few spices is likely to have far-reaching beneficial implication [1]. Curcumin of turmeric (*Curcuma longa*) [2], capsaicin of red pepper (*Capsicum annuum*) [3], fenugreek (*Trigonella foenum-graecum*) [4], garlic (*Allium sativum*) [5] and onion (*Allium cepa*) [6] have been documented to have pronounced hypolipidemic influence in a variety of experimental animal systems, and the latter three spices are found to be efficacious in human studies, too. Prevention and regression of cholesterol gallstone disease by dietary spice principles (curcumin and capsaicin) [7,8] and amelioration of diabetic nephropathy by dietary curcumin and onion by virtue of their hypolipidemic influence [9,10] have been experimentally documented in our laboratory in recent years.

Hyperlipidemic conditions such as those encountered either during continued intake of atherogenic diets or the one found in poorly controlled diabetes mellitus are believed to affect the fluidity of red blood cells [11]. Our recent study has shown that the structural integrity of red blood cells and, hence, the osmotic fragility are affected in hypercholesterolemic situation induced by an atherogenic diet, as a result of alteration in membrane cholesterol/phospholipid (C/P) ratio [12]. We have also evidenced that dietary hypocholesterolemic spices (curcumin, capsaicin and garlic) offer protective influence on this altered fluidity of erythrocytes in hypercholesterolemic condition [13]. We have also recently examined the antioxidant status of erythrocytes of hypercholesterolemic rats and observed that

* Corresponding author. Tel.: +91 0821 2514876; fax: +91 0821 2517233.

E-mail address: ksri@sancharnet.in (K. Srinivasan).

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the depleted intracellular thiols and glutathione content as well as the lowered activity of glutathione reductase in hypercholesterolemic situation were effectively countered by these dietary hypocholesterolemic spices [14].

In the present investigation, we have examined if a high-fat (30%) diet would have any detrimental influence on the structural lipids and on the fluidity of red blood cells in the resulting hyperlipidemic situation and whether hypolipidemic spices in the diet would offer any beneficial protective influence on the integrity of erythrocyte membranes, which are presumably altered in hyperlipidemic situation. Influence of hyperlipidemic condition on erythrocyte membrane lipids, proteins, membrane-bound enzymes (Na^+,K^+-ATPase and Ca^{2+},Mg^{2+}-ATPase) and osmotic fragility of erythrocytes has been investigated in particular. Furthermore, the extent to which dietary spice principles (curcumin and capsaicin) and the spice garlic would offer any beneficial protective influence on the integrity of erythrocyte membranes is examined.

2. Methods and materials

2.1. Chemicals and reagents

Curcumin, the coloring principle of turmeric (*C. longa*), and capsaicin, the pungent principle of red pepper (*C. annuum*), were procured from M/s Fluka Chemie, Switzerland. Garlic (*A. sativum*) purchased from the local market was freeze-dried and powdered. All chemicals used were of analytical grade.

2.2. Animal treatment

Animal experiments were carried out taking appropriate measures to minimize pain or discomfort in accordance with the guidelines laid down by the NIH, USA, regarding the care and use of animals for experimental procedures and with due clearance from the Institute's Animal Ethics Committee. Female Wistar rats (10 per group) weighing 110–120 g housed in individual stainless steel cages were maintained on various experimental diets ad libitum for 8 weeks. The basal diet consisted of (%) casein, 21; cane sugar, 10; cornstarch, 54; NRC vitamin mixture, 1; Bernhart–Tommarelli-modified NRC salt mixture, 4; and refined peanut oil, 10. High-fat diet (HFD) consisted of (%) casein, 21; cane sugar, 10; corn starch, 34; NRC vitamin mixture, 1; Bernhart–Tommarelli-modified NRC salt mixture, 4; hydrogenated vegetable fat, 25; and refined peanut oil, 5. The spice principles/garlic powder were incorporated into the basal diet/HFD, replacing an equivalent amount of cornstarch to give the various experimental diets containing curcumin (0.2%), capsaicin (0.015%) and garlic powder (2.0%). At the end of the feeding period, the rats were fasted overnight and sacrificed on the next morning under light ether anesthesia. Blood was drawn from the heart into heparinized tubes. Liver excised quickly was weighed and stored frozen until various biochemical analyses.

2.3. Osmotic fragility determination

The method described by Dacie and Lewis [15] employed here provided different concentrations of sodium chloride 0.1% to 0.9% in a series of tubes made from appropriate dilutions of 1% sodium chloride–phosphate buffer, pH 7.4, to a final volume of 5.0 ml. Fresh heparinized blood (20 μl) was pipetted into these tubes containing varying sodium chloride concentration. The contents were gently mixed and allowed to stand for 30 min at room temperature. At the end, the contents of the tubes were mixed again and centrifuged at $500\times g$ for 10 min. Absorbance of the supernatant was measured at 540 nm against water blank. The degree of hemolysis is expressed in percentage, where 100% represents full hemolysis. Median corpuscular fragility is defined as sodium chloride concentration (g/100 ml) bringing about 50% hemolysis.

2.4. Preparation of erythrocyte membranes

Erythrocyte membranes were prepared according to Dodgi et al. [16]. To 1 ml of packed erythrocytes was added 39 ml of 5 mM phosphate buffer, pH 8.2. The red blood cells were gently stirred with a glass rod and then centrifuged at 10,000 rpm for 30 min. Fluffy pellet was collected and washed two to three times with 5 mM phosphate buffer, pH 8.2, until a pale pink color is obtained. Finally, this membrane pellet was suspended in the same phosphate buffer and kept frozen until used.

2.5. Extraction of lipids from erythrocytes

Erythrocyte membrane lipid was extracted using the procedure of Rose and Oaklander [17]. Blood collected in heparinized tubes was centrifuged at 2000 rpm for 10 min and washed thrice with 5 volumes of 0.9% NaCl solution for removal of buffy coat. Packed red blood cells (1.0 ml) were pipetted into a 20-ml tube with a Teflon-lined screw cap. Distilled water (1.0 ml) was added, mixed and allowed to stand for 5 min. Isopropanol (11.0 ml) was added slowly with mixing for 1 h. After 1 h, 7.0 ml of chloroform was added and mixed. After 1 h, tubes were centrifuged at $500\times g$ for 30 min. Supernatant was made up to a convenient volume with isopropanol/chloroform mixture (3:2 v/v). Aliquots of this extract were used for cholesterol and phospholipid estimation. Total lipids from plasma and liver were extracted and purified according to Folch et al. [18].

2.6. Lipid analysis

Cholesterol in the lipid extracts from plasma and liver and in erythrocyte membrane was estimated as described by Searcy and Bergquist [19]. Plasma cholesterol associated with HDL fraction was determined after precipitation of apolipoprotein B containing lipoproteins with heparin–manganese reagent according to the method of Warnick and Albers [20]. LDL–VLDL precipitate was extracted with chloroform–methanol (2:1 v/v) and an aliquot was taken for cholesterol determination. Triglycerides in plasma and liver

were determined by the method described by Fletcher [21] using triglyceride purifier (Sigma, USA) to remove phospholipids. Phospholipids were estimated by the ammonium ferrothiocyanate method of Charles and Stewart [22].

2.7. Na^+,K^+-ATPase

Na^+,K^+-ATPase activity was assayed according to Vajreshwari et al. [23] in a medium containing 20 mM Tris–HCl (pH 7.4), 140 mM NaCl, 14 mM KCl, 3 mM $MgCl_2$, 3 mM ATP and 0.2 mM EDTA in a final volume of 0.5 ml, and additionally, ouabain was also included at 1 mM for the assay of ouabain-sensitive enzyme activity. Reaction was started by adding ATP to the preincubated reaction mixture containing suitable aliquot of the enzyme (50–100 μg protein) and incubated at 37°C for 1 h. The reaction was stopped by adding 0.5 ml of 10% TCA and then centrifuged to remove protein precipitate, and the supernatant was used to estimate the released inorganic phosphate (Pi). Pi was estimated according to Ames [24] by adding to 0.3 ml of the sample 0.7 ml of mixture of one part of 10% ascorbic acid and six parts of 0.42% of ammonium molybdate in 1 N sulfuric acid and incubating at 45°C for 20 min. The absorbance was measured at 620 nm and compared with standard 10 to 50 nmol Pi.

2.8. Ca^{2+},Mg^{2+}-ATPase

Ca^{2+},Mg^{2+}-ATPase activity of the erythrocyte membrane was determined according to Vajreshwari et al. [23] in a medium containing 80 mM NaCl, 5 mM $MgCl_2$, 3 mM ATP, 20 mM Tris–HCl (pH 7.4), 0.5 mM $CaCl_2$ and 1 mM ouabain in a final volume of 0.5 ml. Reaction was started by the addition of ATP; the presence of 0.5 mM EGTA without calcium served as the blank. Assays were carried out using 200–250 μg protein. Reaction was stopped by adding 0.5 ml of 10% TCA. Pi was estimated in the supernatant after centrifugation as described by Ames [24].

2.9. Acetylcholinesterase

In erythrocytes, acetylcholinesterase was assayed by spectrophotometric measurement of acetylthiocholine using Ellman's reagent as described by Steck and Kant [25]. To 5–10 μg RBC membrane protein placed in a cuvette was added an equal volume of 0.2% triton X-100 followed by 0.7 ml phosphate buffer (0.1 M, pH 7.5). The reaction was started by the addition of 50 μl of 10 mM DTNB and 50 μl of 12.5 mM acetylthiocholine iodide. Absorption of the colored compound formed was recorded at 405 nm for 5 min.

2.10. Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis of erythrocyte membrane proteins was carried out on 10% acrylamide gel according to Fairbanks et al. [26]. After the electrophoresis, the protein bands were visualized by staining with Coomassie brilliant blue.

Results are expressed as mean±S.E.M. and comparisons between groups were made by means of an unpaired Student's *t* test [27]. Differences were considered significant when $P<.05$.

3. Results

Plasma lipid profile in high-fat fed rats maintained on curcumin, capsaicin and garlic diets is presented in Table 1. Plasma cholesterol essentially remained unaffected in high-fat fed animals. Plasma triglyceride was elevated by about 85–90% in high-fat fed animals. Most of the elevated triglyceride in this high-fat fed rats resided in the LDL–VLDL fraction. Dietary curcumin, capsaicin and garlic effectively countered this In curcumin treatment, the plasma triglyceride value was lowered to a level of 132% of corresponding control. In capsaicin treatment, the elevation in triglyceride was completely reversed. In garlic treatment, plasma triglyceride value was brought down to 130% of corresponding control. Plasma phospholipid content remained essentially unaffected in high-fat treatment.

Lipid profile of erythrocyte membranes of hypertriglyceridemic rats resulting from HFD is presented in Table 2. Cholesterol and phospholipid content of erythrocyte membranes in high-fat fed animals were comparable to those of

Table 1
Influence of spices on plasma lipid profile in high-fat fed rats

Animal group/diet	Triglycerides			Phospholipid	Cholesterol		
	Total	LDL–VLDL	HDL		Total	LDL–VLDL	HDL
Basal control	84.2±3.28	65.8±2.26	18.4±1.35	203.0±7.01	52.3±2.57	23.3±2.19	29.0±1.30
Basal curcumin	84.3±4.13	64.5±2.50	19.8±1.20	199.7±4.02	48.6±1.38	23.0±1.22	25.6±0.58
Basal capsaicin	91.3±4.99	74.9±2.10	16.4±0.12	183.8±4.68	59.6±2.42	33.8±2.60[a]	25.8±0.18
HFD control	144.7±4.11[a]	120.9±5.73[a]	23.8±2.56[a]	201.1±8.65	51.1±1.81	26.9±1.26	24.1±0.83
HFD curcumin	103.2±3.75[b]	75.6±3.52[b]	27.6±3.00	234.9±8.00	53.4±1.64	29.6±1.57	25.8±0.81
HFD capsaicin	78.9±4.49[b]	65.3±4.44[b]	13.7±0.79[b]	213.3±5.57	51.7±2.18	24.4±2.02	27.2±1.02
Basal control	59.7±2.08	51.4±2.01	8.25±0.13	199.9±7.05	53.7±2.78	33.6±1.30	20.1±1.07
Basal garlic	58.7±2.69	51.1±2.60	7.56±0.18	184.6±6.63	52.9±2.95	31.9±1.94	21.1±0.72
HFD control	114.9±4.85[a]	100.2±4.37[a]	15.4±1.19[a]	189.4±5.37	49.2±1.93	26.1±1.80[a]	23.2±1.38
HFD garlic	77.5±3.38[b]	67.9±2.10[b]	9.64±0.40[b]	186.4±3.16	47.7±2.22	24.4±1.74	23.3±1.80

Values expressed as milligrams per deciliter are mean±S.E.M. of 10 animals in each group.
[a] Significantly different from corresponding basal control group.
[b] Significantly different from corresponding HFD control group.

Table 2
Influence of dietary spices on erythrocyte membrane lipids in high-fat fed rats

Animal group/diet	Cholesterol (mg/ml)	Phospholipid (mg/ml)	C/P ratio
Basal control	1.34±0.07	4.41±0.16	0.304±0.018
Basal curcumin	1.39±0.09	4.44±0.21	0.313±0.010
Basal capsaicin	1.55±0.10	4.83±0.25	0.320±0.014
HFD control	1.43±0.08	5.14±0.11	0.278±0.011
HFD curcumin	1.38±0.04	5.03±0.08	0.269±0.008
HFD capsaicin	1.51±0.06	4.97±0.15	0.304±0.009
Basal control	1.58±0.02	5.32±0.15	0.297±0.005
Basal garlic	1.61±0.03	5.47±0.13	0.294±0.006
HFD control	1.56±0.02	5.28±0.14	0.295±0.006
HFD garlic	1.58±0.02	5.43±0.15	0.291±0.004

Values are mean±S.E.M. of 10 animals in each group.

normal ones. As such, the cholesterol to phospholipid molar ratio in red blood cell membranes remained unchanged in high-fat treatment. Spice treatments also did not make any difference on the lipid profile of red blood cell membrane in either normal or high-fat fed rats.

Hepatic lipid profile of dietary high-fat-induced hypertriglyceridemic animals is given in Table 3. Significantly, high total lipid content evidenced in the livers of high-fat fed rats was found to be slightly but significantly reversed by all the three test spices. Hepatic triglyceride value, which was elevated prominently in high-fat treatment, was found to be partially countered by all of the three test spices. Hepatic cholesterol was slightly lower in high-fat fed animals, and this was countered by dietary garlic. Liver weights were also significantly increased in high-fat treatment (Table 4).

The effect of dietary intake of hypolipidemic spices (curcumin, capsaicin and garlic) on the osmotic fragility of erythrocytes of hypertriglyceridemic rats induced by HFD is depicted in Figs. 1 and 2. In normal control rats, hemolysis began in hypotonic solution at 0.5% NaCl concentration and completed at 0.3% concentration, with mean cell fragility (50% hemolysis) being evident at 0.402±0.003% NaCl concentration. In hypertriglyceridemic control rats also,

Table 3
Influence of spices on hepatic lipid profile in high-fat fed animals

Animal group/diet	Total lipid	Cholesterol	Triglycerides	Phospholipid
Basal control	52.4±0.60	4.92±0.23	9.48±0.53	38.0±0.75
Basal curcumin	52.5±0.66	4.99±0.11	8.21±0.73	38.9±0.44
Basal capsaicin	53.7±1.53	5.47±0.26	10.3±0.24	37.9±1.19
HFD control	59.3±0.63[a]	4.19±0.26	15.1±0.48[a]	40.0±0.86
HFD curcumin	52.6±0.72[b]	3.82±0.13	9.30±0.74[b]	39.5±0.96
HFD capsaicin	52.9±0.47[b]	4.00±0.09	8.90±0.71[b]	40.0±0.74
Basal control	46.7±1.21	4.92±0.12	13.3±0.82	34.4±0.83
Basal garlic	46.2±0.82	4.91±0.06	14.4±0.81	36.9±0.73
HFD control	61.5±2.33[a]	4.67±0.07	28.7±2.49[a]	38.2±0.91
HFD garlic	51.4±1.37[b]	5.05±0.09	15.4±1.22[b]	40.9±0.82

Values expressed as milligrams per gram fresh liver are mean±S.E.M. of 10 animals in each group.
[a] Significantly different from corresponding basal control group.
[b] Significantly different from corresponding HFD control group.

Table 4
Influence of spices on liver weights in high-fat fed rats

Animal group/diet	Liver weight (g/100 g body)	Body weight (g)	
		Initial	Final
Basal control	2.64±0.08	110.2±0.71	166.5±2.87
Basal curcumin	2.83±0.08	112.8±1.42	165.0±3.88
Basal capsaicin	2.86±0.08	110.3±2.22	160.0±5.00
HFD control	2.92±0.08[a]	111.9±1.06	184.2±3.12[a]
HFD curcumin	3.07±0.08	111.0±1.22	178.9±2.19
HFD capsaicin	2.86±0.08	114.5±1.38	183.3±3.33
Basal control	2.61±0.07	113.9±1.22	182.1±4.91
Basal garlic	2.66±0.02	114.1±1.28	175.3±4.86
HFD control	2.92±0.11[a]	116.8±1.16	195.4±4.01[a]
HFD garlic	2.76±0.07	116.7±1.23	203.9±3.46

Values are mean±S.E.M. of 10 animals in each group.
[a] Significantly different from corresponding basal control group.

hemolysis of red blood cells began in hypotonic saline at 0.5% NaCl concentration and completed at 0.3% NaCl concentration, but the mean cell fragility (50% hemolysis) was observed at 0.385±0.012% NaCl concentration, which is significantly lower than the corresponding values in the basal control group. Hemolysis expressed as percentage hemoglobin released at 0.4% NaCl concentration as compared with the release at 0.1% NaCl was significantly lower in the HFD control groups compared to the respective basal control groups (36.9±0.11% and 37.1±0.16% vs. 52.8±0.30%). Thus, the osmotic fragility data suggested that red blood cells of HFD fed animals were relatively more resistant to osmotic lysis. The mean cell fragility of red blood cells or the % hemolysis at 0.4% NaCl concentration, which was lower in high-fat fed rats, was partially and significantly restored toward normal by each of the spice treatment in high-fat fed rats.

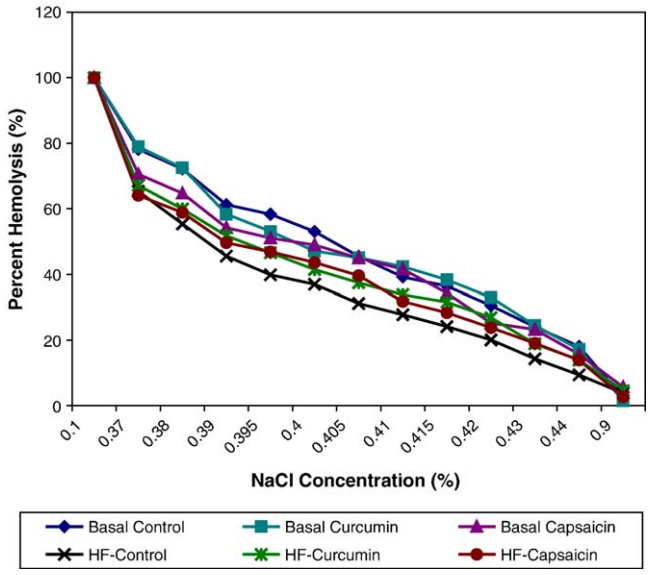

Fig. 1. Osmotic fragility of erythrocytes of high-fat fed rats maintained on dietary curcumin and capsaicin. Values are mean of 10 animals in each diet groups. Standard error of the mean in each group was within 5% of the mean value.

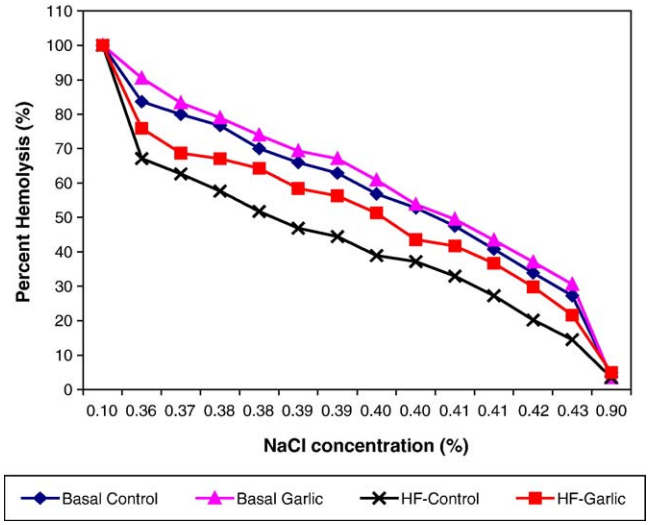

Fig. 2. Osmotic fragility of erythrocytes of high-fat fed rats maintained on dietary garlic. Values are mean of 10 animals in each diet groups. Standard error of the mean in each group was within 5% of the mean value.

The effect of dietary intake of hypolipidemic spices (curcumin, capsaicin and garlic) on the Na^+,K^+-ATPase activity of erythrocytes of hypertriglyceridemic rats induced by HFD is given in Table 5. The total as well as ouabain-insensitive enzyme activities in the erythrocyte membrane were significantly higher in high-fat treatment. Dietary spice principles or garlic did not beneficially influence the altered Na^+,K^+-ATPase enzyme activity in this situation. The ouabain-sensitive component of the total Na^+,K^+-ATPase activity in the hypertriglyceridemic rats remained unaltered.

Influence of dietary spices on the activities of Ca^{2+}, Mg^{2+}-ATPase and acetylcholine esterase in erythrocyte membranes of high-fat fed rats is shown in Table 6. Activity of Ca^{2+},Mg^{2+}-ATPase was significantly decreased in high-fat fed rats (14%). Dietary curcumin, capsaicin and garlic effectively countered the reduction in this enzyme activity. Acetylcholine esterase activity in erythrocyte membrane was unchanged by feeding HFD. The two spice principles or garlic in the diet did not influence the enzyme activity either. Sodium dodecyl sulfate polyacrylamide gel electrophoresis profile of the erythrocyte membrane proteins was unaltered under hypertriglyceridemia, and dietary spices did not influence the protein profile either (figure not shown).

Table 6
Influence of dietary spices on Ca^{2+},Mg^{2+}-ATPase and acetylcholine esterase of erythrocyte membrane in high-fat fed rats

Animal group/diet	Ca^{2+},Mg^{2+}-ATPase [U/(mg h)]	Acetylcholine esterase [μmol/(min mg)]
Basal control	0.232±0.007	156.8±7.35
Basal curcumin	0.239±0.011	169.0±5.51
Basal capsaicin	0.240±0.010	155.5±2.74
Basal garlic	0.212±0.006	157.9±7.95
HFD control	0.201±0.009[a]	160.3±6.13
HFD curcumin	0.222±0.009	150.5±4.07
HFD capsaicin	0.214±0.007	147.4±7.63
HFD garlic	0.228±0.004[b]	153.4±7.49

Values are mean±S.E.M. of eight animals per group.

4. Discussion

The fluidity of the erythrocyte membrane is determined by a number of factors among which cholesterol content, fatty acid composition and protein matrix have significant influences [28,29]. The interaction of these factors seems to influence to a varying degree the physiological properties of the membranes. Erythrocyte membrane composition can be altered by dietary factors. In the present study, feeding an HFD to rats, which essentially produced hypertriglyceridemia, did not alter membrane lipid composition in erythrocytes. We have recently documented that in hypercholesterolemic situation caused by feeding a cholesterol-enriched diet, where the cholesterol content of plasma increased, the concomitantly higher C/P molar ratio in the blood plasma had a direct influence on cholesterol transfer from plasma to erythrocytes, resulting in the accumulation of cholesterol in erythrocyte membrane [12].

Hypertriglyceridemia can be produced in experimental animals by (i) excessive dietary intake of fat, (ii) excessive dietary carbohydrates such as fructose or sucrose, which lead to enhanced lipogenesis, and (iii) reduced clearance from blood or decrease in the uptake of lipids by peripheral tissues. Feeding a 30% fat diet (25% hydrogenated fat +5% vegetable oil) to laboratory rats for 6–8 weeks in earlier

Table 5
Influence of dietary spices on Na^+,K^+-ATPase of erythrocyte membrane in high-fat fed rats

Animal group/diet	Ouabain insensitive specific activity	Ouabain sensitive specific activity	Total specific activity	Ouabain sensitive, % of total
Basal control	0.198±0.006	0.177±0.006	0.375±0.007	47.2±1.40
Basal curcumin	0.200±0.009	0.173±0.006	0.373±0.008	46.4±1.58
Basal capsaicin	0.205±0.002	0.166±0.006	0.371±0.008	44.6±1.55
Basal garlic	0.209±0.008	0.191±0.006	0.400±0.002	47.8±1.49
HFD control	0.227±0.008[a]	0.171±0.007	0.398±0.008[a]	43.0±1.49[a]
HFD curcumin	0.227±0.004	0.170±0.004	0.397±0.006	42.8±1.16
HFD capsaicin	0.228±0.006	0.178±0.004	0.405±0.002	44.0±1.14
HFD garlic	0.232±0.006	0.160±0.008	0.401±0.006	42.1±1.98

Values are mean±S.E.M. of eight animals per group.
Activity units: micromole Pi liberated per hour per milligram protein.
[a] Significantly different from corresponding basal control value.

studies from this laboratory has been documented to result in a significant degree of hypertriglyceridemia [30,31], and hence, the same model was employed in the current study to observe the changes in erythrocytes as a result of experimentally induced hypertriglyceridemia. The significant hypotriglyceridemic effect of the two spice principles and garlic as evidenced in the current study in the dietary high-fat-induced hyperlipidemic rats is consistent with reports of a similar effect in other experimental models. Curcumin feeding has been reported to lower blood triglycerides (both total and HDL associated) in dietary sucrose-induced hypertriglyceridemia [32]. A decrease in serum triglyceride levels is also reported to be brought about by capsaicin feeding in fructose fed animals [33]. Dietary garlic has been shown to decrease hypertriglyceridemia caused by fructose feeding [34].

As evidenced in the current study employing the high-fat fed rats, the liver triglyceride-lowering effect of dietary spice principles and garlic has been reported in other experimental models. Curcumin feeding has been reported to lower liver triglycerides in sucrose fed rats [32]. Capsaicin feeding has been shown to significantly counteract the increase in triglyceride levels in livers of rats fed normal and fructose containing diet [33]. Dietary garlic has been shown to decrease the increased liver triglycerides caused by fructose feeding [34]. It is suggested based on this data that garlic in the diet counteracts the hyperlipogenesis in liver and also increased secretion of VLDL–LDL triglycerides from liver to blood produced by fructose feeding. Sucrose/fructose feeding is known to induce hypertriglyceridemia. Garlic feeding has also been documented to partially counteract the accumulation of triglycerides in liver caused by carbon tetrachloride or ethionine administration, which essentially impedes secretion of triglyceride from liver to blood [34]. It is proposed that garlic partly releases the block in hepatic triglyceride secretion probably either by influencing the lipoprotein formation or by enhancing the secretion of these lipoproteins into plasma.

When there is an alteration in membrane lipid composition, changes in membrane properties are to be expected. We have recently reported that in erythrocytes from rats under alimentary hypercholesterolemia, membrane fluidity was diminished while these cells featured about 20% higher than normal C/P molar ratio [12]. Dietary hypocholesterolemic spices (curcumin and capsaicin) or garlic improved the erythrocyte fluidity in hypercholesterolemic rats, concomitant with the partial restoration of the altered C/P molar ratio in the membrane [12]. Contrary to this observation of mean cell fragility of erythrocytes of hypercholesterolemic rats being higher than normal animals, the same was somewhat lower than that of normal control indicating a slightly increased rigidity in the case of high-fat fed rats here in the current study. Concomitant with this, we did not observe any alteration in the cholesterol and phospholipid content in the membrane of erythrocytes from rats under alimentary hypertriglyceridemia. Such a decrease in mean cell fragility of erythrocytes, or in other words, a slightly increased rigidity of the erythrocytes in high-fat fed rats, has also been reported by others indicating an increase in surface area per volume ratio in erythrocyte membrane [11]. The ability of erythrocytes to deform, one of the most important determinants for their survival in the circulation, involves several factors including the deformability of the membrane itself, the surface area per volume ratio of the cell and the internal viscosity [35]. Osmotic fragility is a determinant of the deformability property of erythrocytes, which is essential for their function and survival against destruction by the spleen. Although in this study, we did not observe any alteration in the cholesterol and phospholipid content and, hence, in C/P molar ratio of erythrocyte membrane in rats under hypertriglyceridemia induced by HFD, the osmotic fragility data still suggested an altered membrane fluidity in these cells as indicated by a slight (nevertheless, significant) resistance of the erythrocytes to osmotic lysis. Dietary spices (curcumin and capsaicin) and garlic were evidenced to beneficially counter the decreased mean cell fragility of erythrocytes and thus restore the normalcy of erythrocyte fluidity in high-fat fed rats.

Membrane abnormalities include increased fragility of erythrocytes, or else, increased resistance to osmotic lysis, altered lipid composition, reduced activity of acetylcholine esterase and increased ouabain-sensitive Na^+,K^+-ATPase. Factors such as younger-cell population, reticulocyte count, cell energy (ATP) depletion, increased C/P molar ratio causing increased area/volume ratio of the cell surface [36] and increased unsaturated fatty acids have been implicated in making the erythrocyte membrane resistant to osmotic lysis [37–40] causing decreased osmotic fragility of the erythrocyte. The magnitude of osmotic resistance of erythrocytes in hypertriglyceridemic rats is such that it may not be simply discounted. In the absence of any change in C/P molar ratio in the erythrocyte membranes caused by hypertriglyceridemic situation, there is a possibility of involvement of other factors in the explanation of increased osmotic resistance of erythrocytes in hypertriglyceridemic rats. The possibility of increased intracellular calcium accumulation, the ATP depletion and the increased C/P ratio are some of the factors examined here to get an explanation to the altered osmotic fragility of erythrocytes in hypertriglyceridemia.

Membrane-bound enzymes are sensitive indices of altered cellular environment, and thus, their study could form one of the approaches to the understanding of the biochemical basis of the pathogenesis. Electrolytes play an important role in the maintenance of structure and function of a living cell. Cell membrane Na^+,K^+-ATPase forms the enzymatic basis of the cation pump activity. The presence of two cation transport pumps in the erythrocytes and other cell membranes has been recognized; one of them that is inhibited by the cardiac glycoside ouabain is an energy-dependent active pump and has received considerable attention. The increased populations of reticulocytes and younger cells may not be the

causative factors for the observed change in osmotic fragility because ouabain-sensitive Na^+,K^+-ATPase, an excellent indicator of cell age and maturation [40], remained unaltered in the HFD fed animals.

Ca^{2+},Mg^{2+}-ATPase is a reflection of energy-dependent calcium transport across the cell membrane. A decreased activity of this enzyme would contribute to the accumulation of intracellular calcium, which will then bind to the inner surface of the membrane and make the membrane less deformable [39]. Impairment of Ca^{2+},Mg^{2+}-ATPase could lead to accumulation of intracellular calcium and has been associated with increased resistance of erythrocytes to osmotic lysis in certain in vitro and in vivo experiments [38,41]. Impairment of Ca^{2+},Mg^{2+}-ATPase could lead to accumulation of intracellular calcium [42]. Inhibition of Ca^{2+}-ATPase in human erythrocytes in *t*-butyl hydroperoxide-induced oxidative stress has been reported [43]. Probably, increased oxidative stress in high-fat fed rats might have contributed to the decreased Ca^{2+},Mg^{2+}-ATPase activity.

Activity of erythrocyte acetylcholinesterase, which is a membrane-bound enzyme, might be affected by changes in membrane fluidity. Erythrocyte membrane acetylcholinesterase, besides Na^+,K^+-ATPase, is also an excellent indicator of cell age [40]. The absence of any alteration in the activity of erythrocyte membrane acetylcholinesterase in the HFD fed animals observed in the present study also suggests that the increased populations of reticulocytes and younger cells may not be the causative factor for the observed change in osmotic fragility. Reduced membrane fluidity is reported to be accompanied by an increase in the activity of membrane acetylcholinesterase in the riboflavin deficient rats [44]. Shinitzky and Barenholz [45] have suggested that a decrease in membrane fluidity may lead in some instances to elevation in the activity of membrane-bound enzymes. *t*-Butyl hydroperoxide that increases acetylcholinesterase activity also increases the erythrocyte membrane fluidity because of increased lipid peroxidation [46].

We have recently observed that total thiols and glutathione in the erythrocytes of high-fat fed rats were depleted significantly [31]. Concentration of lipid peroxides in the erythrocytes (both intracellular as well as membrane) induced by H_2O_2 was significantly higher in the high-fat fed group. Dietary spice principles [curcumin (0.2%) or capsaicin (0.015%)] or garlic powder (2.0%) in the diet were effective in reducing the oxidant stress, which was indicated by significant countering of the depleted intracellular thiols, especially glutathione and elevated lipid peroxides in erythrocytes [31].

In the present study, curcumin and capsaicin have been fed to the experimental animals at a level corresponding to about 10 times the human dietary intake of the respective spices, turmeric and red pepper by Indian population. In the present study, erythrocytes of hypertriglyceridemic rats induced by HFD featured somewhat resistance to osmotic lysis. In the absence of any change in the C/P molar ratio in the erythrocyte membrane, activities of membrane-bound enzymes (ouabain-sensitive Na^+,K^+-ATPase and acetylcholinesterase) and membrane protein profile, a decreased activity of Ca^{2+},Mg^{2+}-ATPase could, have probably contributed to the accumulation of intracellular calcium leading to the diminished deformability of the erythrocytes. Besides exhibiting the hypotriglyceridemic potency, the spice principles (curcumin and capsaicin) and the spice garlic have displayed a protective influence on the erythrocyte integrity in the HFD-induced hyperlipidemia.

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